REMARKS

In the Office Action dated April 22, 2011, claims 74-90, 92-95, 97-107, and 109-112 were pending, of which claims 74-89 were withdrawn from consideration. Claims 90, 92-95, 97-107, and 109-112 were examined and rejected. Specifically, claims 90 and 107 were objected to for alleged informalities. Claims 90, 92-95, 97-107 and 109-112 were rejected under 35 U.S.C. §112, first paragraph, for allegedly lacking enabling support.

This Response addresses the Examiner's rejection and objection, and is believed to place the application in condition for allowance. Accordingly, entry of the Response and allowance of the case are therefore respectfully requested.

Substance of Telephone Interview

A telephone interview was conducted on September 6, 2011 between the undersigned attorney and Examiner Nguyen. The rejection under 35 U.S.C. §112, first paragraph was discussed. The undersigned attorney presented arguments in support of the enablement of the subject claims. The Examiner asked the undersigned attorney to submit the arguments in writing and agreed to reconsider the rejection. The undersigned attorney wishes to thank the Examiner for the courtesy and assistance extended to Applicants during the interview.

Formal Objections

Claims 90 and 107 have been amended, consistent with the Examiner's suggestions. Withdrawal of the formality objection to these claims is therefore respectfully requested.

Rejection Under 35 U.S.C. §112, First Paragraph

The Examiner asserts that the specification is enabling for a genetically engineered strain of *Pichia*, wherein the strain is transformed with a nucleic acid coding for a *full-length T. reesei* α-1,2-mannosidase. However, the Examiner alleges that the specification does not reasonably provide enablement for a genetically engineered strain of *Pichia* transformed with other nucleic acid coding for a *T. reesei* α-1,2-mannosidase to attain the specific desired result.

The Examiner first notes that the breadth of the claims is broadly directed to a nucleic acid coding for a *T. reesei* α-1,2-mannosidase (including but not necessarily limited to a full-length *T. reesei* α-1,2-mannosidase). The Examiner also asserts that at the effective filing date of the present application (June 30, 2000), little was known about a modification of the protein glycosylation pathway in a *Pichia* yeast strain to generate $Man_5GlcNAc_2$ as a predominant N-glycan structure or a predominant intermediate N-glycan structure. In support of his position in this regard, the Examiner refers to several publications that reported failed attempts to generate $Man_5GlcNAc_2$ as a predominant N-glycan structure. The Examiner also refers to a post-filing publication, Choi et al. (2003), which allegedly discloses that a proper length of the α-1,2-mannosidase catalytic domain is one of several factors that determine the yield of $Man_5GlcNAc_2$ in *P. pastoris* Och1 mutant strains. Moreover, the Examiner contends that any modification (even a "conservative" substitution) to a critical structural region of a protein is likely to significantly alter its functional properties. The Examiner notes further that the specification only exemplifies successful results of a full-length *T. reesei* α-1,2-mannosidase, and that a full-length *T. reesei* α-1,2-mannosidase has a pH optimum of 5.0 while most enzymes active in the ER and Golgi have pH optima that are between 6.5 and 7.5, as discussed in Gerngross, US 2002/0137134 A1. Therefore, the Examiner takes the position that it is unclear as to whether any

T. reesei α-1,2-mannosidase that is less than a full-length enzyme would still be stable and sufficiently active in the less than optimal environment of a yeast's ER to produce $Man_5GlcNAc_2$ as a predominant N-glycan structure or a predominant intermediate N-glycan structure.

Applicants respectfully disagree and traverse the rejection based on the following.

The specification illustrates successful results using an enzymatically active fragment of T. reesei α-1,2-mannosidase

The Examiner's conclusion that the specification only exemplifies successful results using a full-length *T. reesei* α-1,2-mannosidase is incorrect. While Examples 2-3 of the specification illustrate successful results as correctly stated by the Examiner, the experiments described in these examples did not involve a full-length *T. reesei* α-1,2-mannosidase. Rather, in Example 2, the genetically engineered *Pichia pastoris* strain was transformed with plasmid pGAPZMFManHDEL. As described on pages 32-33 of the specification, this plasmid includes a fusion between the prepro-signal sequence of the *S. cerevisiae* α-mating factor and a portion of *T. reesei* α-1,2-mannosidase containing its catalytic domain. The sequence of this fusion protein, set forth in SEQ ID NO: 14, is reproduced hereinbelow:

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MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYSDLEGDFDVAVLPFSNSTN
NGLLFINTTIASIAAKEEGVSLEKREAEAEFATKRGSPNPTRAAAVKAAFQTSWNAYHHF
AFPHDDLHPVSNSFDDERNGWGSSAIDGLDTAILMGDADIVNTILQYVPQINFTTTAVAN
QGSSVFETNIRYLGGLLSAYDLLRGPFSSLATNQTLVNSLLRQAQTLANGLKVAFTTPSG
VPDPTVFFNPTVRRSGASSNNVAEIGSLVLEWTRLSDLTGNPQYAQLAQKGESYLLNPKG
SPEAWPGLIGTFVSTSNGTFQDSSGSWSGLMDSFYEYLIKMYLYDPVAFAHYKDRWVLGA
DSTIGHLGSHPSTRKDLTFLSSYNGQSTSPNSGHLASFGGGNFILGGILLNEQKYIDFGI
KLASSYFGTYTQTASGIGPEGFAWVDSVTGAGGSPPSSQSGFYSSAGFWVTAPYYILRPE
TLESLYYAYRVTGDSKWQDLAWEALSAIEDACRAGSAYSSINDVTQANGGGASDDMESFW
FAEALKYAYLIFAEESDVQVQATGGNKFVFNTEAHPFSIRSSSRRGGHLAHDEL*
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The portion corresponding to the prepro-signal sequence of the *S. cerevisiae* α-mating factor is underlined, and the portion representing the fragment of *T. reesei* α-1,2-mannosidase is

highlighted. By comparing to the full-length *T. reesei* α-1,2-mannosidase, it is clear that this fusion protein did not include the N-terminal 24 amino acids of the full-length *T. reesei* α-1,2-mannosidase. The first 20 amino acids at the N-terminus of the full-length *T. reesei* α-1,2-mannosidas are believed to include a hydrophobic sequence which serves as a signal peptide (see, Maras et al., *J Biotechnol* 77:255-63, 2000, submitted with the Information Disclosure Statement filed on September 25, 2003).

State of the Art Regarding T. reesei α-1,2-mannosidas

Regarding the alleged lack of information and failed attempts in the art with respect to production of $Man_5GlcNAc_2$ as a predominant N-glycan structure or a predominant intermediate N-glycan structure in a *Pichia* yeast strain, Applicants respectfully submit that this observation by the Examiner simply confirms the novelty and unobviousness of the claimed invention. The publications cited by the Examiner do not support a position that the state of the art with respect to *T. reesei* α-1,2-mannosidase itself was unpredictable. In contrast, Applicants respectfully submit that at the effective filing date of this application (June 30, 2000), *T. reesei* α-1,2-mannosidase had been well characterized in terms of its functional domains, along with other members of α-1,2-mannosidases (MS-I).

Most MS-I enzymes are known to be localized in the Golgi or endoplasmic reticulum, although a few are secreted and have extracellular activity. See, Gonzalez et al., *Mol Biol Evolution* 17:292-300 (2000) (copy attached); for example, the bridging paragraph at pages 294-295. The topology of those enzymes that localize to the ER and the Golgi includes a luminal catalytic domain and an N-terminal transmembrane region. See, Herscovics, *Biochimie* 8: 757-62 (2001) (copy attached). The transmembrane region is composed of a stem region (closest to

the luminal catalytic domain) a transmembrane domain, and a cytoplasmic tail, although not all members contain each of the three segments. In the secreted MS-I enzymes, the extra-catalytic transmembrane region is also known as a leader sequence, serving as a signal for secretion of the enzyme. The *T. reesei* α-1,2-mannosidase belongs to the category of secretory MS-I's along with those of *A. saitoi* and *P. citrinum*, as described by Gonzalez et al., *supra*, and Maras et al., *supra*. Secreted MS-I enzymes are composed of a catalytic domain and an N-terminal leader sequence.

The precise divisions between domains are somewhat arbitrary and may vary among different groups of researchers. However, they often do not vary by more than a few amino acids. The catalytic domains of these MS-I enzymes contain regions of high similarity across species compared to other regions of the proteins. For example, Becker et al. (*European J. Cell Biol* 79: 986-992 (2000); copy attached) studied the MS-I enzymes from mouse and *S. cerevisiae*, and identified the catalytic domain of the former protein (MS-IB) as amino acids 171-641 and the latter, amino acids 23-549 (see page 3, right column, 4th paragraph). Schneikert and Herscovics (*Glycobiology* 4: 445-450 (1994); copy attached) reported that the catalytic activity of a murine MS-I was not affected by removal of 105 N-terminal amino acids, concluding that the N-terminal 25% of the protein is not essential for enzyme activity (see page 448, left column, 3rd paragraph). Gonzalez et al. (*J. Biol Chem* 274: 21375-86 (1999); submitted with the Information Disclosure Statement filed on July 8, 2009) examined several MS-I enzymes. Gonzalez et al. (1999) found two in *C. elegans*, one of which had the catalytic domain from amino acid 91-531, the other from amino acid 89 to 532. Gonzalez et al. (1999) also found the catalytic domain of the human MS-I as having amino acid 220 to 663. Gonzalez et al. (1999) additionally placed the catalytic domain of the *S. cerevisiae* MS-I (from the ER) from amino acid 45 to 549. See Table I, Figure 4, and pages 21379, right column to 21380, right column of Gonzalez et al. (1999). Maras et al., *supra*,

predicted the first 20 amino acids of the *T. reesei* enzyme to include a hydrophobic signal peptide for secretion, and fused a heterologous secretion signal to a portion of the *T. reesei* enzyme between amino acids 25 and 523 (see page 259, right column, first paragraph), apparently believed to represent the catalytic domain of the enzyme.

It is clear from these publications that while the beginning and ending amino acids of the catalytic domains of various MS-I enzymes as defined by different research groups may vary, those skilled in the art have the ability to determine the catalytic domain of a particular enzyme.

Applicants also direct the Examiner's attention to the alignment of amino acid sequences of several α-mannosidases (including *T. reesei* α-1,2- mannosidase), which was provided with Applicants' Response filed October 9, 2009 and is provided again herewith as Exhibit 1. All of the sequences were available at the time the present application was filed. Such sequence alignment reveals a very clear homology among α-mannosidases, especially at the C-terminal region, which facilitates a determination of the conserved catalytic domains of these proteins (shown by shaded area). Further, *in vitro* assays were available and described in the art (e.g., U.S. Patent 5,834,251, Examples 7-8 on colns. 24-25) which would permit an easy comparison of the activity of a particular fragment of *T. reesei* α-1,2- mannosidase with that of the full-length protein.

In view of the foregoing, Applicants respectfully submit that those skilled in the art would be able to readily ascertain whether a fragment of *T. reesei* α-1,2- mannosidase encompasses its catalytic domain and therefore retains the enzymatic activity of a full-length *T. reesei* α-1,2- mannosidase for purposes of the present invention.

Post-Filing Art – Choi et al. (2003) and Gerngross, US 2002/0137134 A1

Turning to Choi et al., the Examiner states that this reference discloses that a proper length of the a-1,2-mannosidase catalytic domain is one of several factors that determine the yield of $Man_5GlcNAc_2$ in *P. pastoris* Och1 mutant strains. However, there was no actual data in Choi et al. showing the differences in length of the "catalytic domains" employed or the effect of the different lengths on the yield of $Man_5GlcNAc_2$. The reference mentions that the experimental protocol and data from the experiment would be published at a later date, and were indeed published in 2011 (Nett et al., *Yeast* 28:237-252, 2011; a copy is attached herewith).

The experiments described by Choi et al. and Nett et al. aimed to test a large variety of targeting sequences for their abilities to target an MS-I molecule to an intracellular organelle membrane. Therefore, a heterologous targeting sequence was fused to an MS-I molecule lacking a portion of its native N-terminal transmembrane region. Notably, the *T. reesei* MS-I was not included in the studies of Choi et al. or Nett et al. As described in Nett et al., with one exception (Δ387 on page 344), all of fusion constructs used in the experiments included the entire catalytic domain of the respective MS-I molecule. The constructs differed from one another, however, in the remaining length of the transmembrane region of the relevant MS-I molecule and the length of the heterologous transmembrane region domain used in the chimeric fusion constructs. See, e.g., Figure 1 of Nett et al. That is, the studies of Nett et al. (hence the studies of Choi et al.) do not provide any basis in support of the position that the yield of $Man_5GlcNAc_2$ was affected by the varying lengths of the catalytic domains of the MS-I enzymes. Moreover, it is also clear from Nett et al. that the catalytic domains of the MS-I enzymes were intended to be used in their entirety to make the studies therein meaningful, and that the catalytic domains of the MS-I enzymes could be readily ascertained.

Turning to the Gerngross patent publication, this publication allegedly discloses that a full length *T. reesei* α-1,2-mannosidase has a pH optimum of 5.0 while most enzymes active in the ER and Golgi allegedly have pH optima that are between 6.5 and 7.5. Therefore, the Examiner takes the position that it is unclear as to whether any *T. reesei* α-1,2-mannosidase that is less than a full length enzyme would still be stable and sufficiently active in the less than optimal environment of a yeast's ER to produce $Man_5GlcNAc_2$ as a predominant N-glycan structure or a predominant intermediate N-glycan structure.

From a careful reading of paragraph 68 in Gerngross which the Examiner has relied upon, one would note that the statement in that paragraph is only accurate for *S. cerevisiae* and is based on the authors' perceived dependence of activity on pH for the enzymes in the ER or Golgi. While references are provided by Gerngross for the pH optima of various enzymes, there is no confirmation by Gerngross that the pH in the ER or Golgi of *Pichia* is the same as that of *S. cerevisiae* or that the pH optima of enzymes in the *Pichia* ER/Golgi are in the same range. In fact it is well known that *Pichia* grows at a wider range of pH values than *S. cerevisiae*. *Pichia* can grow between pH 3.0 and pH 7.0. See Higgins and Cregg, *Methods in Mol Biol* 103 *Pichia Protocols*, Higgins and Cregg (eds.) 1998, p10 (copy attached). *Pichia* are often cultured at pH 6.0. Therefore, Applicants respectfully submit that the Examiner's position that other less than full-length *T. reesei* α-1,2-mannosidase molecules would not be stable or sufficiently active is not substantiated, especially given the successful results demonstrated in the present application using a catalytic domain-containing yet less than full-length *T. reesei* α-1,2-mannosidase in *Pichia*.

In view of the foregoing, Applicants respectfully submit that the subject matter as claimed is fully enabled as required by 35 U.S.C. §112, first paragraph. Reconsideration and withdrawal of the enablement rejection are therefore respectfully requested.

Conclusion

It is firmly believed that the subject application is in condition for allowance, which action is earnestly solicited.

Respectfully submitted,

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Enc.: IDS, citing supporting references not previously made of record; Exhibit 1.